
Supplementing the Immune System with Plant-Produced Antibodies

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Antibodies are inherently stable proteins found in all mammals and in fish. They have high specificity and low toxicity as potential therapeutics. In the drug industry today, they have much higher approval rates through the clinical process than do new chemical entities, small molecules. They have been formulated into injectable, topical and oral applications. They are appropriate for chronic conditions because they are relatively long-lived in the body and they have some potential long-lasting benefits as supplements to the immune system.

Secretory immunoglobulin A (IgA) and immunoglobulin G (IgG) are present in the human body in great abundance. The IgGs circulate in blood serum, whereas the IgAs are secreted by sebaceous glands and across almost all of the epithelial tissues, the oronasopharyngeal cavities, the pulmonary tract, and the gastrointestinal and urogenital tracts. Each of us makes in the vicinity of 2.5 g of IgA every day, most of which pass into the environment in one way or another. IGAs and IgGs constitute much of the protein component that is found in milk in mammals.

They are our natural defenses. Circulating antibodies protect against invading organisms, including viruses and bacteria, and also against toxins that are produced by ourselves and also by a variety of organisms with which we come in contact, including parasites, fungi, bacteria, and viruses. Secretory antibodies are our first line of molecular defense against invading organisms. These organisms populate the mucosal surfaces, which, because they are warm and wet, are conducive to the growth of bacteria and viruses. The presence of secretory IgAs prevents the colonization or even attachment of those organisms, and thereby prevents subsequent infection or invasion parenterally into our bodies.

¹Now with Chromatin, Inc., Chicago, IL.

Passive antibodies are received by infants when breast-fed. Colostrum is a rich source of antibodies, secretory IgAs as well as IgGs, and it is the first bolus of antibodies that infants receive. They absorb the antibodies through the epithelium of the mouth and via the gastrointestinal tract into the circulatory system. Each of us makes in the vicinity of 10 million different antibodies at any given time. No two people make exactly the same antibodies, partly due to the fact that the immunome, the genome of our immune system evolves with time as we are exposed to various antigens and depends on our state of health.

ANTIBODIES ON THE MARKET

Antibodies are commercially available. They first appeared on the market in the mid-1980s. Called OKT3, it was used for organ-transplant rejection, but these molecules have a much broader array of disease indication. ReoPro® is for coronary stenosis. Retuxin is a non-Hodgkin's lymphoma anti-cancer molecule. Remicade® is an anti-inflammatory molecule. Synergis is an anti-infection molecule that is used in premature infants to prevent infection of the pulmonary tract with respiratory syncytial virus. And Herceptin® is a breast-cancer therapy.

Ten antibodies are on the market today, for organ-transplant rejection, cancer therapy, cardiovascular disease, epithelial infections, and inflammatory disease. A number of molecules are in clinical use for systemic infections and there are molecules in preclinical development to deal with oral and topical prophylaxis, primarily for infectious diseases. And several antibodies have been added to over-the-counter applications that are not related to infectious disease but are for prevention of a number of disorders.

PRODUCTION SHORTFALL

The therapeutic markets that have the pharmaceutical companies enthusiastic—because there are large clinical unmet needs for which payer-providers are willing to reimburse—are, primarily, inflammatory CNS disease, cardiovascular disease, and infectious diseases.

The problem is that there is a production shortage; some antibody drugs are production-limited. Irwin Goldman mentioned the anti-inflammatory drug Enbrel®, which is used for rheumatoid arthritis. The manufacturers are unable to produce enough Enbrel® to meet market demands because manufacturing facilities are relatively expensive to construct, they take a long time to build and then they need to be validated by the Food and Drug Administration (FDA). Demand for these products is projected to exceed capacity by a factor of as much as four by 2005; in 3 years we may be able to produce only half, or less, of the antibody molecules that the market will demand. Manufacturing facilities can take 5 years to build and be validated, therefore the choice to build or not build has to be made before knowing whether the drug will get through the process of proving safety and efficacy to the FDA, and be a commercial success.

Factory construction for a successful molecule costs about half a billion dollars. The cost of the pharmaceutical in the marketplace is \$500 to \$1000/g, which is what most of them actually cost to manufacture. That means that the therapy cost to the individual or payer-provider for a chronic disease is probably in the order of \$10,000 to \$15,000. Enbrel® costs around \$10,000 to 12,000/year. Some of the anti-cancer molecules are of a similar cost for a course of acute therapy. Such high costs face resistance from payer-providers, but certainly if, as a consumer, you chose to use it yourself it would also be prohibitive. There is a significant cost challenge to using these molecules.

SYNTHESIS IN CROP PLANTS

One of the solutions that seemed obvious to us as plant biologists was to use plants, which are highly efficient producers of proteins. Plants assemble and secrete very complex proteins of their own, and after transfer of the appropriate genes with the right regulatory sequences into plant systems they can synthesize and assemble functional antibodies. The endomembrane system in plant cells is very much like our own. The plant cell recognizes the signals on an antibody molecule and effects assembly in the same way as an animal cell—the same way as human B cells or plasma cells. And then of course we know that there are significant excess crop capacity in the United States that could provide high-value pharmaceuticals like antibodies. The required acreage would probably be less than 5% of the entire acreage for any of major crops such as soybean or corn. There is plenty of capacity.

Many plants and plant tissues have been shown to assemble antibodies with transgenic proteins. We use corn and direct the synthesis of these molecules into the endosperm. The endosperm is made up of cells that are fundamentally similar, therefore, we use a promoter that expresses genes in those cells so that the molecule is made by a single cell type or a small number of cell types that are similar.

When a murine protein is synthesized in a plant is it the same as the original? If you put an antibody gene in a plant, make the antibody and extract it, what does it look like? Does it look like a human-synthesized antibody? The answer is: pretty much. The peptide sequence is identical, and the molecule is folded and assembled and put together the same way. It binds to its target antigen exactly as it should—we now have about forty examples of that.

We can also make those complicated secretory IgA molecules in plants, the synthesis of which in the human body requires two different cell types. In contrast, we can convince the plant cell to make the molecule and assemble it in a single cell, which is more efficient.

One area in which differences exist between plant- and human-synthesized proteins is in post-translational processing. Antibodies are glycoproteins; sugars are attached at various amino acids in the backbone chain of the protein, and those sugars, or glycans, are characteristic for different systems. Mammals

use slightly different glycosylation pathways from plants. Although the backbone and the core glycans are identical the terminal glycans are different.

At this stage, we do not know enough in terms of impact of these differences on efficacy and safety. That information is part of what the FDA demands when a drug is approved. We do know that if you make a mouse antibody in a plant and put it back into a mouse then there is no antigenicity, there is no allergic reaction in the mouse. Also, the circulating half-life of the molecule and the pharmacokinetics or biodistribution are identical in the mouse for mouse- and plant-synthesized molecules. These data indicate that the plant-synthesized molecules are safe and will be treated much like the molecules that we have in our own bodies.

ADVANTAGES WITH CROP-PLANT PRODUCTION

Why should we use plants? They have several advantages over the cell-culture and transgenic animal systems that are available today. Capital costs and operating costs are lower, which are considerations that make the pharmaceutical manufacturers happy, and should also make consumers happy, because these molecules might actually be affordable for a variety of diseases. The material costs are somewhat lower, given that agricultural inputs are relatively inexpensive. Also we can scale up very rapidly. It doesn't take 5 years to build a synthesizing facility. It only takes one season to grow out more acreage of the crop as long as the processing facility can handle the extra material. And, we can scale up very quickly to meet demand, which differs from the situation in the pharmaceutical industry where it takes 4 to 5 years. For instance if Enbrel® were plant-synthesized today, there probably would be a plentiful supply.

In addition, unlike transgenic mammals or cell-culture systems, plants have the advantage of not harboring viruses or replicating viruses that are infectious to humans. There are a number of other zoonotic and prion contaminants that, to this point, have not been found to exist in plant systems, so there are potential safety benefits as well.

REGULATORY CHALLENGES

Regulatory challenges are of greatest concern: are antibodies made in plants—"plantibodies"—fundamentally safe? It depends on two things. First of all, it depends on the antibody itself; is it inherently safe? Secondly, has the antibody been altered by the plant, to make it inherently unsafe? And the only difference we have been able to identify is in the glycans; so the question that is being asked today, and fairly so, is: is the glycan suitable for injection? We know that plant glycans are suitable for topical applications because we are exposed to them throughout our lives and there are no known examples of the glycans on plant proteins being deleterious to humans. Actually, we are well adapted to dealing with those complex glycans.

The regulatory challenge is the clinical trial. To test one of these molecules costs in the order of \$100,000,000 to move all the way through a phase-3 clinical trial. If you are going to invest that resource you must be sure that you have a product that is going to be safe and effective. Furthermore, finding that resource is a challenge.

The FDA and United States Department of Agriculture (USDA) regulatory processes for this new technology are not completely vetted at this point. We await a document from the FDA that has been several years in the making to provide guidance to the industry for the manufacture and clinical evaluation of plant-based biopharmaceutical proteins. It is important that we understand and have good working relationships with the FDA and the USDA in order to move these molecules through the approval process.

FIRST CLINICAL TRIAL: HERPES

Our first clinical trial, which we hope to start in spring or early summer 2003, will be of an antibody that neutralizes all of the known street strains of herpes simplex 1 and herpes simplex 2 viruses. Herpes is one of the most prevalent diseases in North America; over 50 million Americans are infected with herpes simplex 2. About a million and a half new cases are reported annually in the United States. The antibody that we have, is efficacious in an animal model both in the treatment and in the prevention of the disease, so we have two potential uses for the product. To treat a reasonable proportion of the 50 million sufferers, or to prevent the 1.5 million cases, a lot more antibodies will be needed than are available today for treatments such as for breast cancer or for non-Hodgkin's lymphoma or even for rheumatoid arthritis. Using corn, we have the ability to make large quantities at a scale and cost that will be appropriate for the marketplace.

THE FUTURE

There are a number of targets for which antibodies are being developed by Epicyte and by other companies. Largely we are looking at infectious diseases. There are antibodies that recognize beta-amyloid and have a positive impact in Alzheimer's cases; and for oral health there are at least two organisms for which neutralizing antibodies are known to have positive effects on dental caries and gum disease and could probably be used as preventatives.

Our overall goals and challenges have nothing specifically to do with the fact that we are manufacturing molecules in a plant system. They have to do with the overall process involved in bringing a safe and effective drug to the market. Fundamentally, we believe the advantage that plants can bring is that these therapies can be cost-effective and scaleable to the extent that they could be used by the general public. Of course in order to achieve that, we need to make certain that we are protecting the safety and integrity of the food and feed

chains and also of the environment. These issues are part of the discussion that is ongoing with the USDA on guidelines for growing transgenic crops that synthesize pharmaceuticals.

In our view, the most important role at this early stage in the discussion is to inform the stakeholders of the benefits and the risks. People are not well informed of the benefits and some of the risks have not been well assessed, and that is part of the process of forming effective stakeholder partnerships. It includes the regulatory agencies as well as people who will eventually be involved in manufacturing and distributing these drugs.

Some 400 antibody molecules are in preclinical evaluation across biopharmaceutical and biotechnology companies and in academic organizations, and over a hundred are in or beyond phase-1 clinical trials. In ten years I think there will be more than fifty antibody products on the market, and—if we can use plant-based systems—we might see closer to a hundred. We will see a new generation of immunoglobulins, of which Enbrel® is an example (an immunoglobulin backbone with a soluble receptor glued to the end). We will see much lower-cost antibodies both as prescription and as OTC products.

We can succeed, but we have to find effective antibodies, we have to engage the regulatory agencies in order to make certain that these drugs are safe and effective. The agencies make their decisions based on sound science and the onus is on us to provide that. We need to focus on public benefits and risks, practice good stewardship of the environment in terms of the crop systems that we are using and, of course, we need to communicate with all stakeholders.